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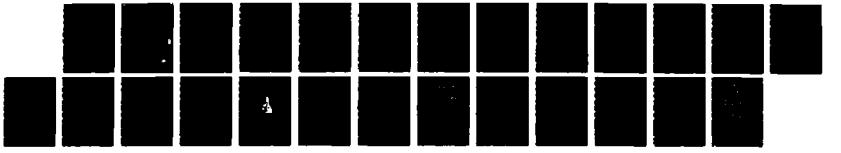

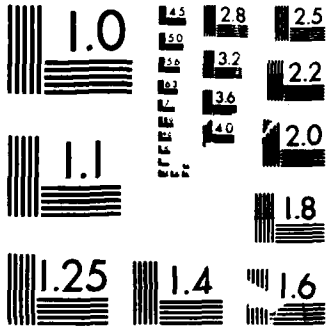

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APPLICATION OF NEW TECHNOLOGIES TO RADIATION BIODOSIMETRY IN MAMMALIAN SYSTEMS

Volume II

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NOTICES

This final report was submitted by the School of Medicine and Dentistry, University of Rochester, Rochester, New York, under contract F33615-84-C-0603, job order 7757-04-37, with the USAF School of Aerospace Medicine, Human Systems Division, AFSC, Brooks Air Force Base, Texas. Captain Jeffrey C. Wigle (USAFSAM/RZB) was the Laboratory Project Scientist-in-Charge.

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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Various procedures, including flow cytometry analysis, premature chromosome condensation (PC techniques, deoxyribonucleic acid (DNA) damage and repair kinetics, and high resolution micr scopic image analysis (Cell Analyzer System), have been used to determine radiation damage produced by low doses of ^{137}Cs. Peripheral lymphocytes isolated from whole-body irradiated mice were stained with Iq and Thy-1.2 immunofluorescence antibodies and analyzed with cyto- meter to determine the cell number and changes in subpopulations (T- and B-cells) as a func- tion of postirradiation time. The results indicated a dose-dependent decrease of lymphocyte [illegible] during the period of 5-day postirradiation time. Cell survival curves constructed fr [illegible] subpopulations of lymphocytes in the dose range of 0-2 Gy showed a linear relation

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19. ABSTRACT (Continued)

ship between radiation dose and log surviving cell number in different subpopulations of lymphocytes, indicating the radiation sensitive nature of the lymphocytes. The D_o value calculated from T- and B-cells showed that B-cells (D_o = 0.75 Gy) are more sensitive than T-cells (D_o = 1.2 Gy). The sensitivity of detecting radiation dose using lymphocyte count could be in the range of 0 to 0.5 Gy when the sensitive B-cell subpopulation was monitored. Radiation-induced chromosome repair measured from Chinese hamster ovary (CHO) cells demonstrated a direct relationship between residual chromosome fragments and cell survival when analyzed by the Cell Analyzer System. Similar results have also been obtained by Pantelias and Maillie (4) in human lymphocytes where the number of chromosome fragments/rad was 3 times higher than those measured from CHO cells. The DNA damage and repair kinetics were measured from in vitro and in vivo mouse lymphocytes, CHO cells, and human squamous carcinoma A431 cells to compare the cellular radiosensitivity with DNA repair capacity. Quantitativity similar amounts of initial DNA damage were produced among the different kinds of cells. However, the repair of DNA strand breaks was significantly faster in A431 cells than CHO and lymphocytes.

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INTRODUCTION

With the increasing exploration of space, the principal hazards to be anticipated during prolonged manned flights in space are those arising from interaction between the space environment and the biological organism.

Of particular importance in this respect are the effects of cosmic radiation on mammalian tissue. An astronaut could possibly receive 1-2 Gy of deposited radiation energy during a long-term space mission. Although this dose is already greater than 20-40% of the LD_{50} dose in humans, the seriousness of the hazard depends upon the particular tissue which has been exposed and the nature and dose rate of the ionizing radiation.

Organs in which radiation damage might be expected to be especially interesting for risk assessment are the peripheral lymphocytes. It is well known that peripheral lymphocytes are most sensitive to radiation damage, abundant in number, and nondividing during the lifetime. Therefore, these cells are most suitable for measuring the effects of low doses of radiation.

After ionizing radiation, mammalian cells can repair radiation damage through mechanisms linked with the repair of chromosome damage and the repair of deoxyribonucleic acid (DNA) damage. The recovery or the survival of dividing tissue may ultimately be correlated with chromosome and DNA repair. However, it is not known if similar mechanisms also operate on nondividing lymphocytes. In this research, we have studied the dose response relationship between organ radiosensitivity, cellular radiosensitivity, the primary lesions which are responsible for cell death and their potential in the assessment of radiation hazard. Combined techniques of flow cytometry, premature chromosome condensation (PCC), alkaline elution, and a Cell Analyzer System have been applied in mouse peripheral lymphocytes, Chinese hamster ovary (CHO) cells, and A431 human cell cultures to establish a sensitive risk index from low doses of ionizing radiation for biodosimetry.

METHODS AND EXPERIMENTAL APPROACH

Culture Conditions of A431 Human and CHO Cells

Human A431 cells were originally established by Dr. Girard at Dr. Todaro's laboratory at the National Cancer Institute in 1973 (1). The biopsy specimen was obtained from an 85-year-old female patient. Pathological diagnosis of the tumor was epidermoid carcinoma of vulva. The explant method was used initially for culturing tumor specimen. The specimen was washed with growth medium and then minced with scalpels to obtain pieces approximately 2 mm in diameter, and 12-15 pieces were then evenly dispersed over the surface of T-30 Falcon flasks. The tissue was placed at room temperature for approximately 15 min to allow attachment and was then covered with 2 ml growth medium.

The cultures were incubated at 37 °C and observed daily for evidence of growth. After significant growth, cells were subcultured after removal from the surface of the flasks with 0.1% trypsin. For routine maintenance of the cultures, Dulbecco's modification of Eagles' minimal essential medium, supplemented with 20% heat-inactivated fetal calf serum, 200 units/ml penicillin, and 200 µg/ml streptomycin, was used as growth medium. Cells were subcultured twice a week by seeding 5 x 10^5 trypsinized A431 cells into 75 cm^2 flasks containing 15 ml complete tissue medium. Cultures were maintained at 37 °C, 100% humidity, 5% CO_2 + 95% air atmosphere. The doubling time of the culture was around 24-30 h.

Human A431 cells were used in this study to compare the DNA damage and repair between human tumor cells and peripheral lymphocytes. Exponentially growing A431 cell cultures were used in all experiments. The endpoint of evaluating DNA damage and repair was the amount of DNA single-strand breaks measured by alkaline elution techniques.

Chinese hamster ovary cells were maintained in F-10 tissue culture medium (GIBCO Laboratory, Grand Island, New York) supplemented with 10% fetal calf serum. These cells were subcultured 3 times a week by seeding 2 x 10^5 trypsinized cells into 25 cm^2 flasks. Exponentially growing cells were used for all experiments. Cell survival measured by colony-forming efficiency and chromosome aberrations determined by PCC methods were the endpoints used in this report.

Analysis of Radiation Effects on Peripheral Blood Lymphocytes

The changes in the numbers and types of cells in the peripheral blood have been assessed following low doses of radiation (0-2 Gy). This method enables us to establish dose-response curves for the individual types of cells, and to determine the postirradiation time when the maximum effects are present.

Groups of 3 mice each were irradiated with 0, 0.5, 1, and 2 Gy of ^{137}Cs gamma rays. On the day of the analysis, mice were bled from the tail vein after the vein was dilated through the use of heat. The blood from the 3 mice in each group was pooled. Red blood cells (RBC) were lysed by adding 0.3 ml of blood to 50 ml of hypotonic lysing solution, and were centrifuged for 20 min at 200 revolutions per minute (rpm). The resulting white blood cells (WBC) were washed over a calf serum gradient to eliminate "ghost cells" and debris. At this point, the cells were divided into two groups. Group 1 was used for staining and analysis for Ig, Thy-1, and $H2D^d$ using flow cytometry, and group 2 was used for analysis of mitogen stimulation indices. Mitogen stimulation was done for 48 h, followed by a 12 h ^3H-thymidine pulse labeling.

Repair of Radiation-Induced Chromosome Damage Measured by PCC Assay

Using the PCC assay, the formation and repair of chromosome damage has been determined using CHO cells and mouse spleen lymphocytes. The residual chromosome damage measured from different radiation doses was assigned as chromosome damage indices.

Exponentially growing CHO cells and lymphocytes isolated from mouse spleen were irradiated with 0, 1, 2, 3, 4, and 5 Gy of ^{137}Cs gamma rays. Following irradiation, cells were incubated for 2, 4, 6, 13, and 24 h to allow chromosome repair. At the end of each repair time, cells were trypsinized and fused with nonirradiated mitotic cells to produce PCC. The number of chromosome fragments was scored microscopically at different postirradiation times.

Measurement of Low-Dose Radiation Survival Curves using the Cell Analyzer System

To study the effect of low-dose radiation on mammalian cells, both surviving cells and inactivated cells should be identified to establish an accurate survival curve. In general, survival of cells following irradiation is measured by the ability of single cells to form colonies under ideal growth conditions. However, the conventional colony-forming assay does not provide a satisfactory result in the lower dose (less than 4 Gy) largely due to systematic errors of counting, dilution, and plating procedures used in the preparation of single-cell suspensions. One way to improve this approach is to locate microscopically individual cells attached to a culture flask, and then observe and classify the cell growth. With this approach most systematic errors are eliminated, and by measuring both the killed cells and the surviving cells, the statistical accuracy is improved. The process of locating and classifying cells by hand is very tedious and time consuming since large amounts of data with good reliability are required for statistically and biologically significant results. An alternative method is to use an automated system.

For this purpose, we have purchased a prototype automatic Cell Analyzer System developed by British Columbia Cancer Center and CombiTech Development, Inc., Vancouver, B.C., Canada. With this system, irradiated cells plated in the tissue culture flasks containing growth medium can be located accurately and their positions are recorded automatically on the first day after plating. On subsequent days, the system revisits the cells located within 100 μm of the first position so that the same cell can be observed and classified (by colonies) easily.

The Cell Analyzer System consists of an inverted microscope (Nikon Diaphot), a modified precision microscope stage (Semprex M5000), two stepping motors (American Superior Electric DR103788-G9, Slo-Syn M061-FD08), translator module (Slo-Syn STM-103), high resolution video camera (Dage MTI, Model NC-65), microcomputer (IBM XT), joystick (T.G. Product), and printer (Epson FX-100+). A complete system block diagram is shown in F gure 1. The following various components and their functions are described:

Microscope. The core of the Cell Analyzer System is a high-quality Nikon Diaphot inverted microscope. Besides the binocular viewing of the microscope image, a video camera has been adapted for recording and displaying of the image.

Precision Stage and Drivers. The flask containing the cells is fixed on an X-Y precision microscope stage. The stage is driven by two stepping motors over a range of 7x12 cm. Each stepping motor is linked to the stage

by a lead screw and a driving nut that translates the rotational motion into linear motion. A single step (1.8° rotation of the stepping motor) translates into 12.5 μm stage movement. The stepping motors are driven by a translator module that provides current to each of the four motor windings in the correct sequence, allowing motion of the stage by one step in either direction.

Computer Control. The IBM XT microcomputer activates the translator modules through an 8-bit parallel output to provide (a) stage control functions (position, velocity) to the joystick position, and (b) program-made selection (scanning, manual, or classification mode) to an array of four push buttons. A flow chart of LDALOC (for locating and recording cell positions) is shown in Figure 2.

Figures 3, 4, and 5 demonstrate the extracted features of the signals related to the physical characteristics of the cells in terms of light scatter, refractive index and optical density, and their geographical location in the flask. These features, when coupled with mathematical transformations of the signals, can not only evaluate the ability of cells to proliferate, but also discriminate cells from preprogrammed features.

DNA Repair in Irradiated Mouse Lymphocytes

Studies have been initiated to measure the DNA damage and repair in lymphocyte cell subpopulations. To overcome the problem of labeling these nondividing cells with radioactive DNA precursors, a fluorometric DNA alkaline elution technique has been developed. As a continuous effort to determine the molecular mechanisms for different radiation sensitivities in subpopulations of lymphocytes, several experiments have been designed for the following studies:

- Repair kinetics of irradiated in vitro spleen lymphocytes
- Repair kinetics of irradiated in vivo spleen lymphocytes
- Relationship between cellular and molecular radiosensitivity of lymphocytes to CHO and human A431 squamous carcinoma cells.

Mouse spleens were removed surgically after the animals were euthanatized by cervical dislocation, and placed into a 15-ml centrifuge tube containing 10 ml of complete Basal Medium-Eagle with 10% fetal calf serum. Single cell suspensions were prepared by placing the spleens between the frosted ends of two glass slides and rubbing the tissue gently. After removing the cell debris, spleen cells were counted and an appropriate number of lymphocytes ($1x10^6$ cells/ml) was placed into a 25 cm^2 tissue culture flask for irradiation and repair study. Single cell suspensions of mouse spleen lymphocytes, CHO, and A431 cells were irradiated with 6 Gy (1.1 min irradiation time) ^{137}Cs gamma rays at room temperature. Immediately after irradiation, these cells were transported for repair to a 37 °C incubator with 100% humidity and 95% air plus 5% CO_2 atmosphere. After irradiation and appropriate repair time, cells were spun at 1500 rpm for 10 min to remove tissue culture medium. The cell pellet was resuspended, and washed with cold (0 °C) phosphate buffered saline three times before loading for alkaline elution assay. Repair times

of 5, 15, 30, 60, 120, and 240 min were chosen to determine both fast and slow repair processes.

The DNA alkaline elution profiles were measured with a fluorometric analysis method. Samples are collected via the standard alkaline elution technique with the following two exceptions:

(1) 5 x 10^6 cells are lysed on 46-mm polyvinylchloride (PVC) filters

(2) 10 ml of ethylenediaminetetracetic acid wash and PVC lysis solutions are used in the loading procedure.

Spectrofluorometric Assay Protocol

Aliquots (1 ml) of DNA-containing samples 1 through 10, and the prewash, are transferred from the elution fraction collector into small glass test tubes. The pH of the samples is neutralized by the addition of 0.2 M KH_2PO_4 and standard citrate saline (pH = 7.00). Finally, 1 ml of Hoechst 33258 (1.5×10^{-6}M) in citric buffer saline is rapidly added to the samples in the dark; the tubes are mixed, and allowed to stand for at least 10 min before analyzing.

To measure the amount of DNA remaining on the filter, the filter is minced and put into a beaker containing 10 ml of 0.3N NaOH. This beaker is allowed to set on a rocker for 4-5 h. The liquid is removed and neutralized with 0.5 N HCl and 0.2M KH_2PO_4. A 1-ml aliquot is transferred to a small glass test tube and 1 ml of citric buffer saline is added along with 1 ml of Hoechst reagent.

The 5 ml wash fraction is neutralized with 0.5N HCl and 0.2 M KH_2PO_4; it is then treated like the filter sample.

RESULTS

Low-Dose Cell Survival Curves Measured from CHO Cells Using the Cell Analyzer System

A typical signal measured across the approximate center of a CHO cell is presented in Figure 3. The cells were plated 2 h before the measurement, and allowed to attach to the plastic surface of the tissue culture flask by placing the flask in a 37 °C incubator. The size and shape of cells do not change detectably for several hours under present measuring conditions. Besides the cell size, shape, location, and numbers, other morphological parameters are also recorded and analyzed for cell survival, nuclear-to-cytoplasmic ratio, and cell movement (Figs. 4 and 5).

A population of 1000 CHO cells was measured with the Cell Analyzer System after irradiation of 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 Gy of ^{137}Cs gamma rays. The distributions of cell number and cell location were recorded repeatedly at 0-, 2-, 4-, 6-, and 10-day postirradiation time. Using a computer program incorporated with the Cell Analyzer System, cell survival

determined from individual cells was constructed. Figure 6 presents a dose response curve of CHO cells measured by the Cell Analyzer System after low doses of ionizing radiation. A similar cell survival curve determined by the conventional dilution-plating technique is also shown in Figure 6 for comparison. The cell survival curve measured by the Cell Analyzer System in the low-dose region has a similar shape to that measured by the conventional method.

The advantages of using the Cell Analyzer System for the direct measurement of cell survival have already been recognized (2, 3). We have shown that the Cell Analyzer System has a better sensitivity in the detection of low doses of ionizing radiation, requires a smaller number of cells (<1000) than conventional dilution and plating methods, and can measure the cell survival in live form without fixing and staining the cells. Besides the proliferating activities, the cell viability of nondividing cells, such as lymphocytes, can be monitored by their mobility in the tissue culture medium measured by the Cell Analyzer System (3).

In our study, the cell survival curve determined by the Cell Analyzer System (Fig. 6) showed a smaller error bar (standard error) in every dose point than that measured by conventional methods, indicating a better statistical probability of detecting low-dose radiation using the Cell Analyzer System.

Analysis of Radiation Effects on Peripheral Lymphocytes

The flow cytometric analysis of mouse lymphocytes indicated that (except for a temporal increase on the second day after irradiation) a reduction of the number of peripheral lymphocytes occurred 1 day after irradiation and remained at the same level for 5 days post irradiation (Fig. 7). Also, a clear dose response of total WBCs and different types of lymphocytes (Thy-1 and Ig positive) has been established in the low-dose range of 0-2 Gy ^{137}Cs gamma ray (Fig. 8).

In Figure 8, we can sensitively detect a radiation dose as low as 50 rads in a dose-dependent fashion simply by looking at the number of different types of lymphocytes in the peripheral blood at a given time after irradiation. These differences are enhanced by flow cytometric analysis of either immunoglobulin bearing cells or Thy-1 bearing cells (Fig. 8). Based on D_0 values calculated from the dose response curves in Figure 7, it is demonstrated that Thy-1.2 positive cells (D_0 = 1.2 Gy) are almost twice as radioresistant as Ig positive cells (D_0 = 0.75 Gy).

The significant findings in this experiment were: (a) measurement of radiation damage using peripheral lymphocytes as biodosimeters can be made at a postirradiation time as long as 5 days after irradiation, and (b) subpopulations of peripheral lymphocytes express different radiation sensitivities; Ig positive cells are more sensitive to radiation than Thy-1.2 positive cells as determined by cell loss measurement.

Chromosome Damage and Repair in CHO Cells

Using the PCC assay, we have measured the formation of chromosome damage and its repair in CHO cells. After 5 Gy of ^{137}Cs gamma rays, the excessive chromosome fragments/cell decreased from 35 fragments/cell immediately after irradiation to 6.3 fragments/cell at 12 h postirradiation incubation (Fig. 9). The repair kinetics curve shows an initial fast repair process in the first 4-6 h followed by a slower repair process. The half times for the fast and slow repair processes are 1.5 and 6 h, respectively. When the residual chromosome damage measured from the slow repair process is plotted against radiation dose, a dose response curve for chromosome damage in CHO cells is obtained (Fig. 10). Although there is a substantial overlap in the number of chromosome fragments produced at lower doses of radiation, detection of radiation damage with the minimum dose of 0.5 Gy is still possible with the PCC technique.

DNA Repair in Irradiated Mouse Spleen Cells

The preliminary results show that the amount of DNA single strand breaks produced in mouse spleen lymphocytes was proportional to the radiation dose received (Fig. 11) whether the spleens are irradiated *in vivo* or *in vitro*, indicating similar molecular damage to that observed previously in the CHO cells. However, after a single dose of 16 Gy, the rejoining of DNA single strand breaks was much less in vitro than in vivo (Fig. 12). At 2 h post-irradiation, in vitro spleen cells still contained 30% of the initial DNA damage compared to only 15% unrepaired damage measured from in vivo conditions.

To compare the DNA repair capacities of cells with different radiation sensitivities as determined by a colony-forming assay, alkaline elution analysis was used in mouse spleen cells, CHO cells, and human A431 squamous carcinoma cells. When the dose response curve was determined by plotting initial elution slopes vs. radiation dose (Fig. 13), a linear relation was observed between DNA strand breaks and radiation dose received.

There was no difference in the initial amounts of DNA strand breaks measured among mouse spleen, CHO, or A431 cells. However, DNA repair studies (Fig. 14) showed a significant difference in repair rate for both fast and slow repair processes. At 120-min repair time, the percentage of DNA damage remaining for A431, CHO, and mouse spleen cells was 6%, 16%, and 30%, respectively, indicating a good correlation with their cellular radiation sensitivity.

DISCUSSION

With the improved flow cytometric analysis and method of removal of RBCs from the sample, we have demonstrated that peripheral lymphocytes can be used as a very sensitive biodosimeter for measuring low doses of ionizing radiation. Using a specific immunofluorescence marker for T- and B- cells, the results indicate that the depression of peripheral lymphocytes occurs at 1 day after irradiation and the level of cell loss remains constant for at least 5 days after the mouse received total body irradiation of 0-2 Gy of ^{137}Cs gamma ray (Fig. 8). There is also a dose-dependent response among different types of lymphocytes. When the responses of subpopulations of lymphocytes are analyzed with Thy-1.2 (T-cells) and Ig markers (B-cells),

the results obtained from B- lymphocytes show 1.8-fold higher radiation sensitivity than that measured from T-lymphocytes.

Since the relative loss of cells from the peripheral blood after irradiation remains constant, survival curves can be constructed from the average numbers of cells remaining in the peripheral blood during the 5-day postirradiation period against the radiation dose received (Fig. 7). Radiobiological parameters calculated from the survival curves show all three curves have an extrapolation number (n) value of approximately 1, indicating the radiation-sensitive nature of the lymphocytes. The D_0 value of B-cells is 0.75 Gy which is about 1.8 times more sensitive than T-cells (D_0 value, 1.3 Gy). Total white cells have an intermediate D_0 value of 1.0 Gy. Based on the linear nature of the survival curves, radiation damage below 0.5 Gy can be readily detected from the loss of subpopulations of lymphocytes at times as long as 5 days postirradiation.

The potential of using peripheral lymphocytes as biodosimeters is indicated since: (a) measurement is time independent; (b) linear response is found between cell loss and radiation dose (0-2 Gy) in all subpopulations of lymphocytes; and (c) B-cells are a more sensitive biodosimeter than T-cells.

Chromosome damage and repair in the forms of breaks could be detected in CHO cells at doses of 0.5 Gy using the PCC method (Fig. 9). Data obtained from the remaining chromosome damage as a function of radiation dose (Fig. 10) indicates a curvilinear relationship at doses below 3 Gy which appears to correspond to the shoulder region of the CHO survival curve (Fig. 6). A complete linear relationship between chromosome damage and radiation dose would not be expected in CHO cells due to the repair potential of the cells after low-dose irradiation; nevertheless, a linear chromosome damage index is still obvious at doses above 3 Gy. The value obtained for number of fragments/rad in CHO cells is about 0.012. By considering the yield of excess chromosome fragments per cell (Y) as the effect of initial radiation injury, the results presented here show that Y is fitted by a linear dose effect relationship, $Y = X + \beta D$, where X is the initial chromosome number, β is one slope, and D is the radiation dose. Besides the results obtained from CHO cells, a similar linear relationship between chromosome damage and radiation dose has been reported by Pantelias and Maillie (4, 5, 6) using mouse and human lymphocytes in the dose range between 0 and 3 Gy. The value of excess chromosome numbers/rad reported by their study is about 0.03 fragment/rad, which is approximately 3 times more sensitive than the results we obtained from CHO cells. Therefore, the dose-response relationship obtained in this work and others with lymphocytes shows that yield of excess chromosome fragments/cell is a sensitive and dose-dependent indicator of radiation damage that can be used to measure the response of this biological dosimeter over a wide range of doses.

To use colony-forming ability as a parameter for biodosimetry, it is important to have an accurate measurement in the low-dose region. The application of a fully automated cell image recognition system would allow a precise measurement of the proliferating ability of each single cell. Using the Cell Analyzer System, cell survival curves of CHO cells were obtained after low doses of radiation (Fig. 6). When using the Cell Analyzer System,

the statistical variation as indicated by the standard error is smaller in the survival curve than when using the conventional dilution plating method. Thus, differences in effects between low doses can be discriminated using the Cell Analyzer System technology.

The DNA damage and repair as measured by alkaline elution has been used to determine the molecular mechanisms of radiation damage and the relationships between cellular radiation sensitivity and DNA repair capacity. The results obtained from this study suggest that human and rodent cells which are more radioresistant as determined by colony-formation assay also express greater and more rapid DNA repair than mouse spleen lymphocytes. Further experiments are required to refine the radiation sensitivity assays for human lymphocytes with PCC and alkaline elution techniques.

In conclusion, using combined technologies of flow cytometry, PCC, the Cell Analyzer System, and alkaline elution, our results provide evidence that the number of different subpopulations of peripheral lymphocytes and the yield of chromosome fragments observed in the interphase cells after ionizing radiation can be used as very sensitive biodosimeters for measuring low doses of radiation damage. According to the procedures described in this report, radiation injury may be detected from the blood sample during long periods after exposure to ionizing irradiation. The dose response relationships measured from the changes in lymphocyte subpopulations, excess chromosome fragments, and cell survival in both CHO cells and lymphocytes suggest that the changes in the number of white cells in blood samples and chromosome damage can be used for prediction of cell survival.

Our future research will involve the improvement of detection sensitivities using lymphocytes as biodosimeters, developing new methods for assessing the survival characteristics of lymphocytes, and measurement of relative biological effectiveness with high linear energy transfer radiation. Some approaches will be: (a) to study the chromosome damage and its dose response relationship from subpopulations isolated by flow cytometry; (b) to improve the chromosome damage detection system using the Cell Analyzer System and high power image analysis of chromosome structure; and (c) to assess the viability of irradiated lymphocytes by their cellular mobility monitored by the Cell Analyzer System.

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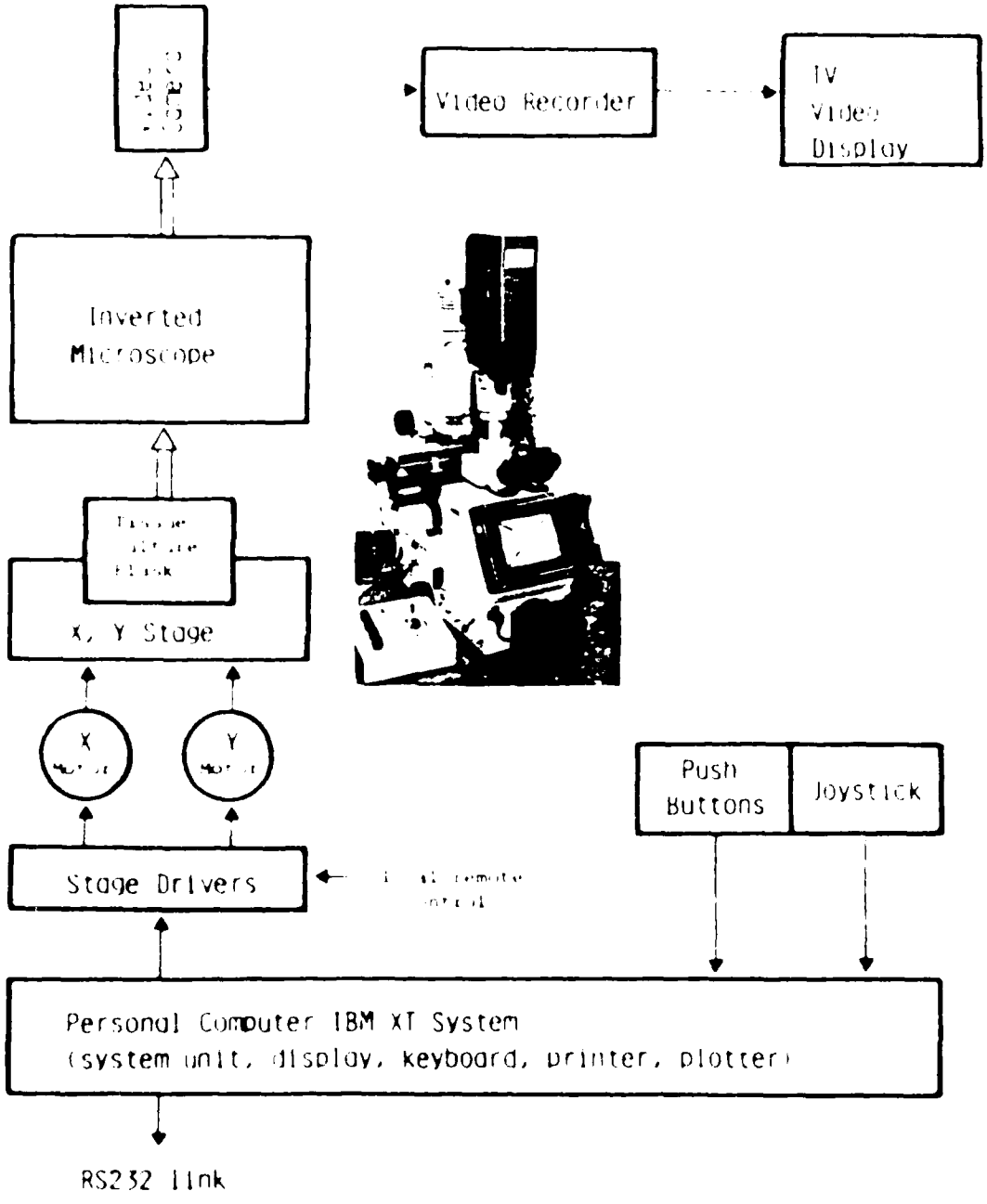

Figure 1. Block diagram of complete Cell Analyzer System.

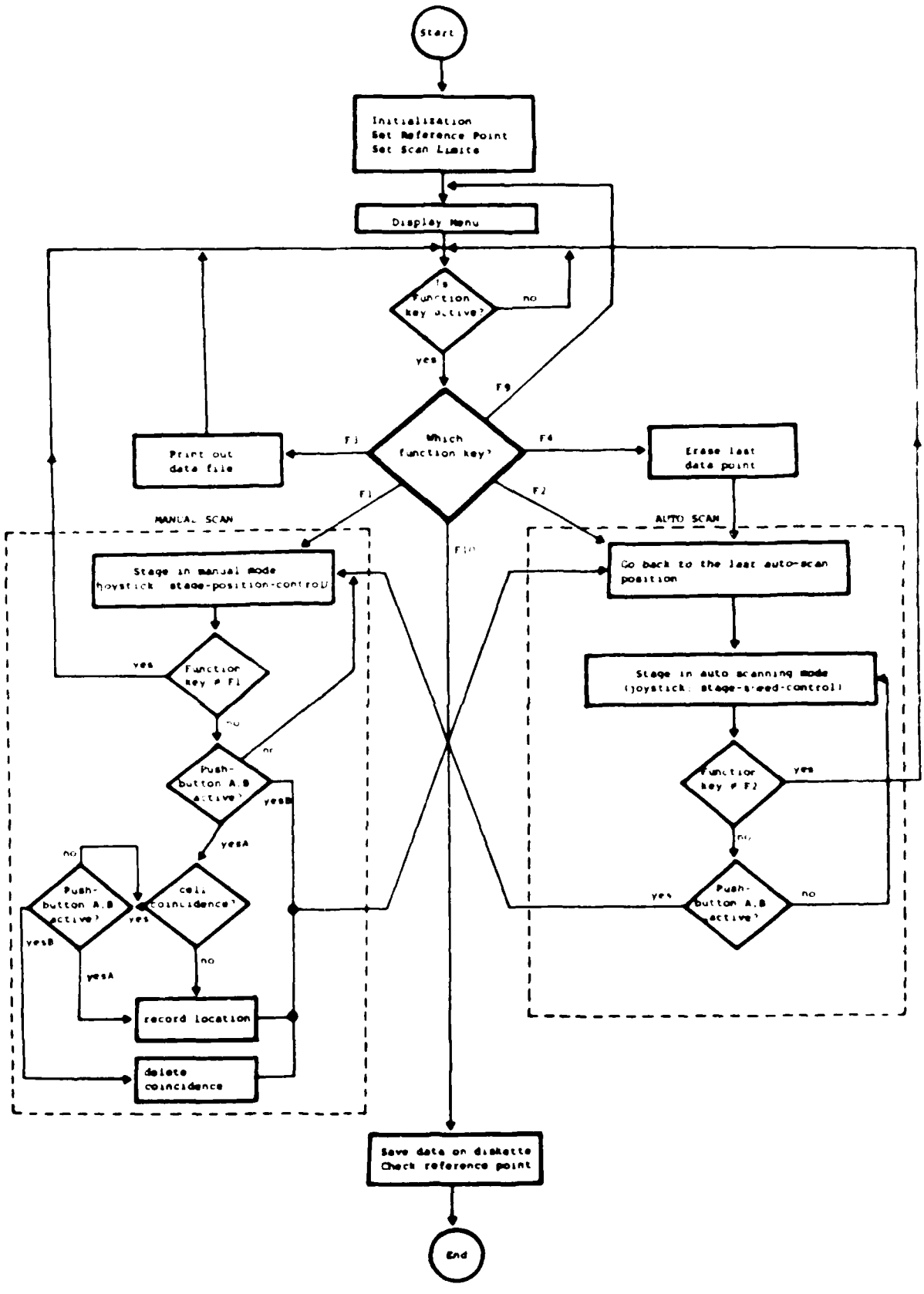

Figure 2. Flow chart of LDALOC computer program used in Cell Analyzer System.

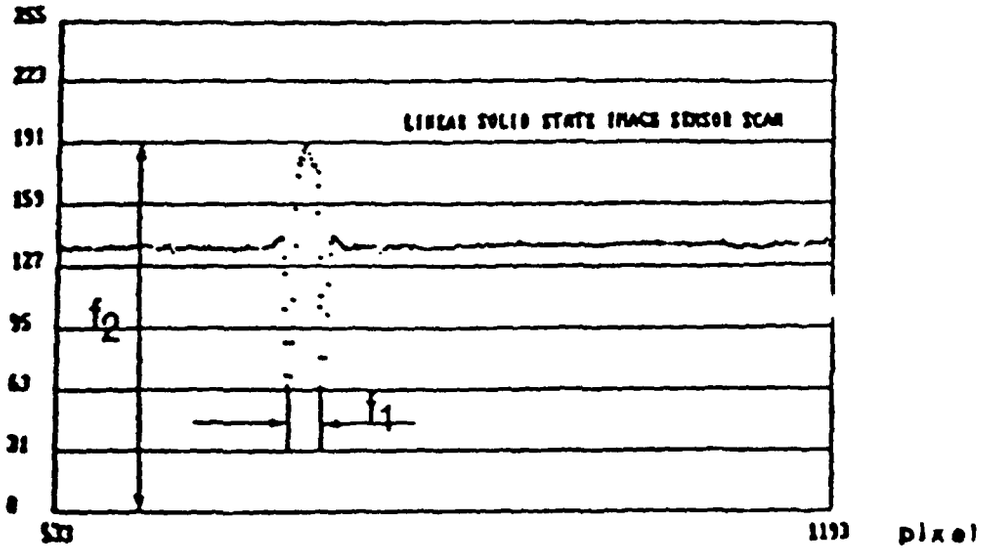

Figure 3. Scanning characteristics of CHO cells determined by Cell Analyzer System. Relative light transmission on the Y axis represents the thickness of the cell, and relative distance in the X axis represents the diameter of the cell. f_1 and f_2 are the minimum boundary conditions used by the computer to discriminate cells from debris.

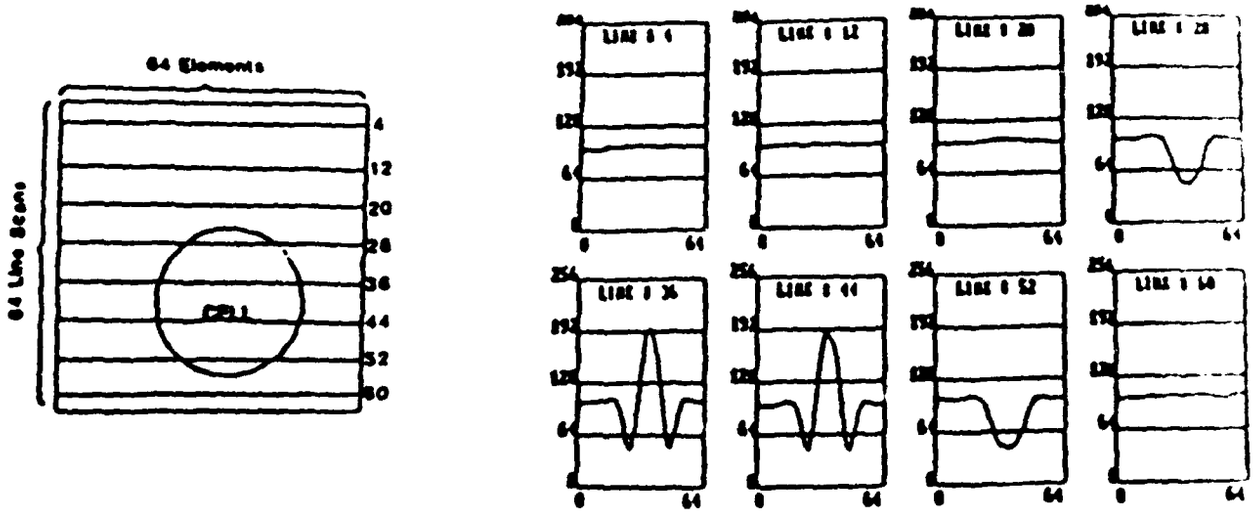

Figure 4. Histogram distributions of different physical parameters measured from a CHO cell for scanning slices at approximately 2-μm intervals. Each block diagram on the right represents the scan for the numbered lines in the diagram on the left.

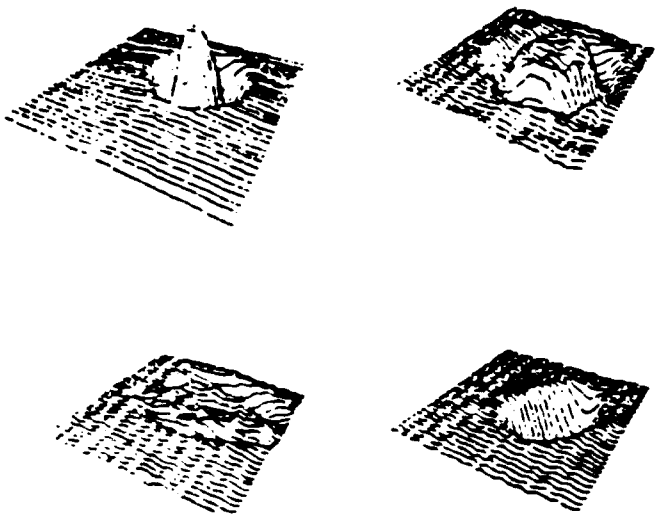

Figure 5. Contour map of a single CHO cell scanned by Cell Analyzer System. The different contour diagrams represent images of the cell scanned from different angles.

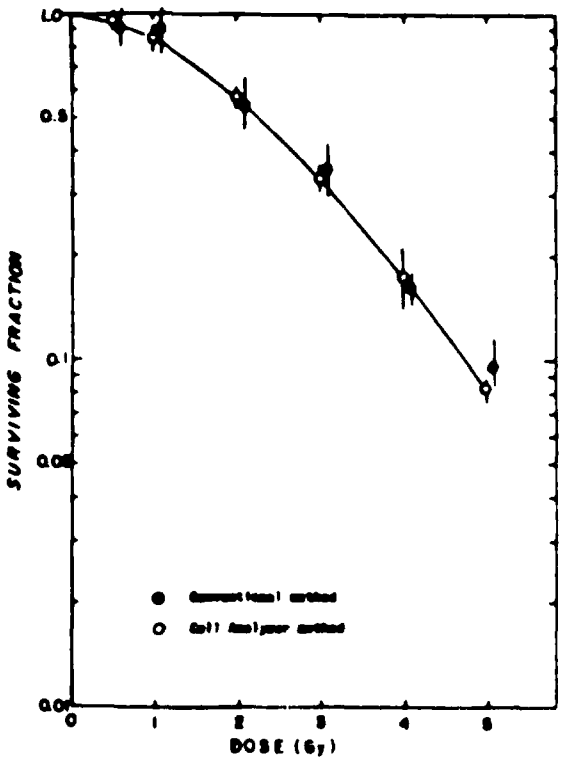

Figure 6. Dose response curve of CHO cells determined by both Cell Analyzer System and conventional dilution plating technique.

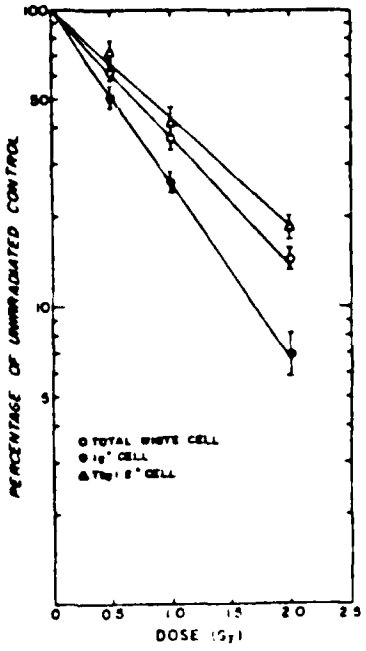

Figure 7. Dose response curve of mouse peripheral lymphocytes after irradiation with 0.5, 1, and 2 Gy of ^{137}Cs gamma ray. The surviving fraction was determined as the average fraction of cells present following a 5-day postirradiation period in vivo. The D_0 doses measured from the individual peripheral blood subpopulations are total white cell: 1 Gy, Ig positive cell : 0.75 Gy, and Thy 1.2 positive cell : 1.25 Gy

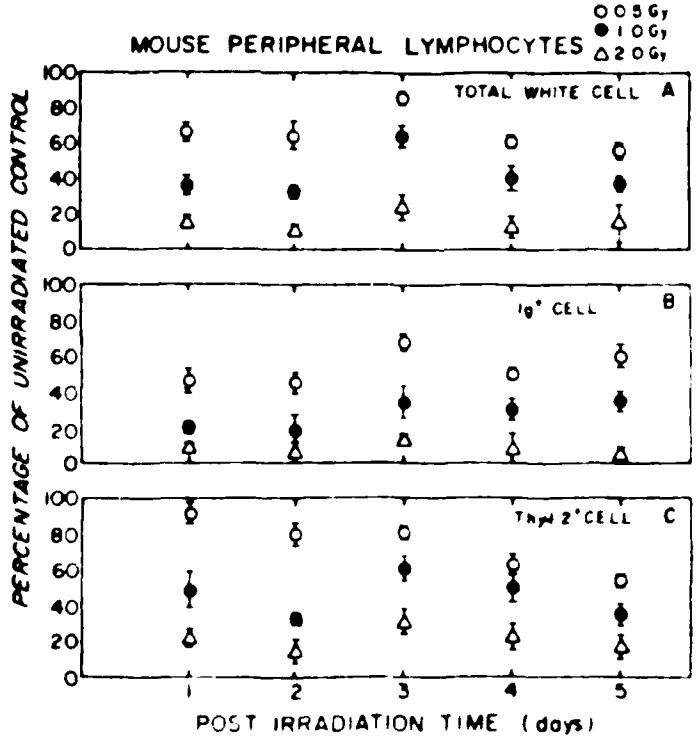

Figure 8. Effects of low dose ionizing radiation on the number of peripheral lymphocytes determined from whole body irradiated mice. The results represent triplet experiments from each measurement.

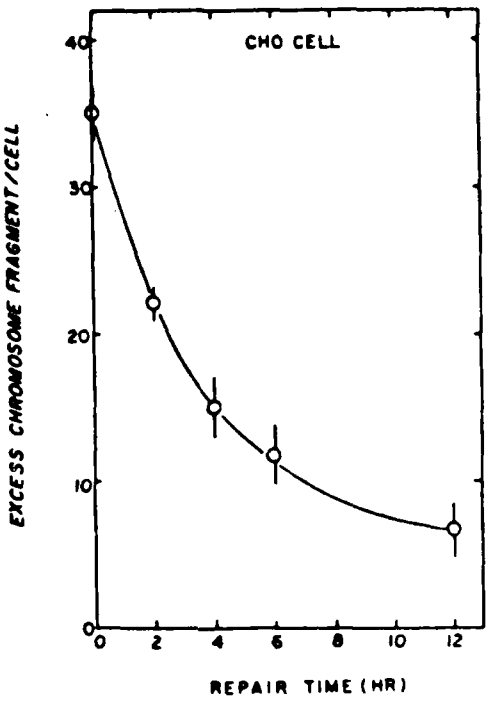

Figure 9. Repair of chromosome fragments in CHO cells after irradiation with 5 Gy ^{137}Cs gamma rays. Excess chromosome fragments/cell was determined microscopically using PCC techniques.

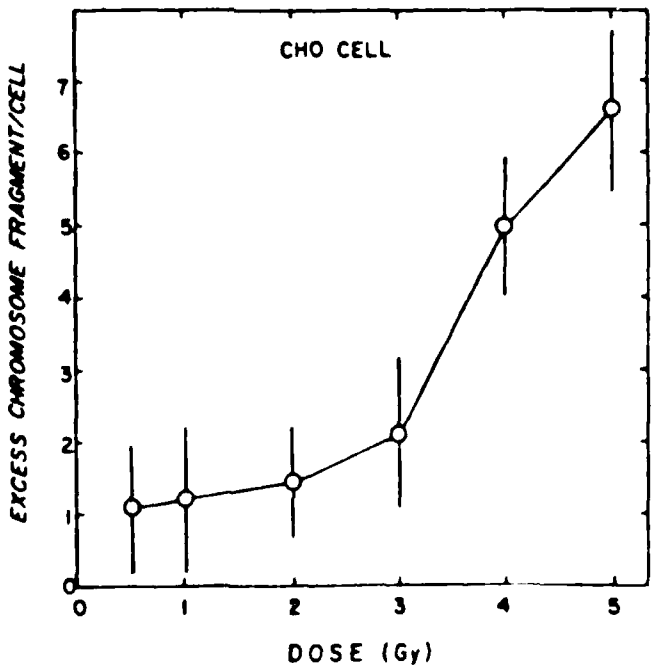

Figure 10. Dose-response curves of residual chromosome damage measured at 12 h postirradiation in CHO cells.

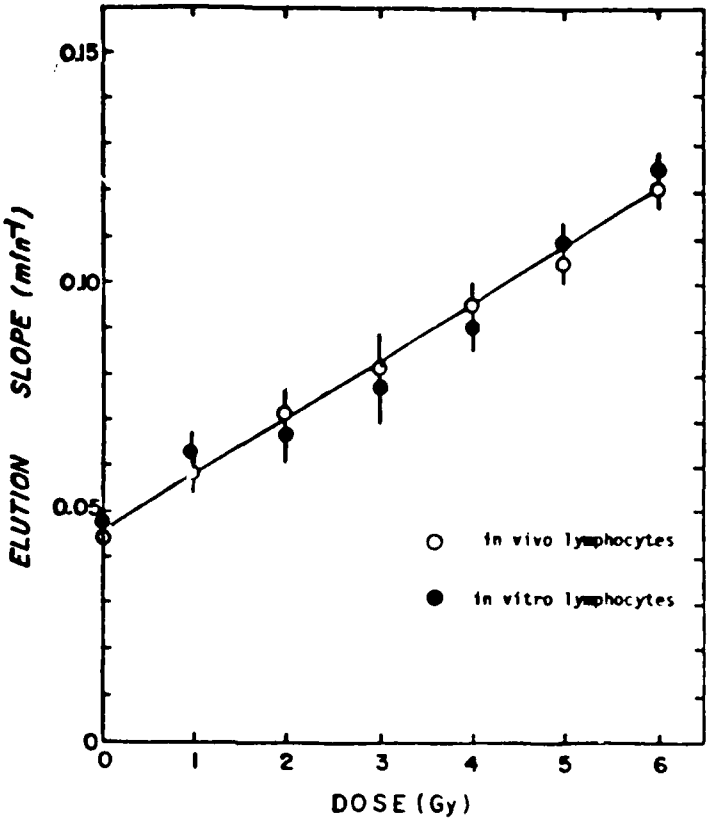

Figure 11. DNA strand break induction efficiency determined from in vivo and in vitro mouse lymphocytes using alkaline elution technique.

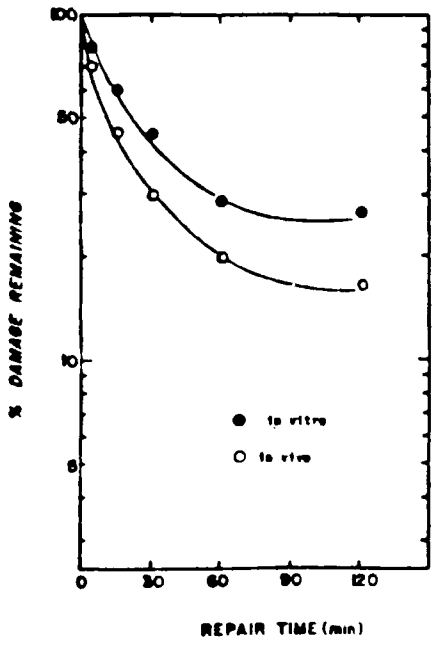

Figure 12. DNA strand break rejoining kinetics determined from [illegible] lymphocytes.

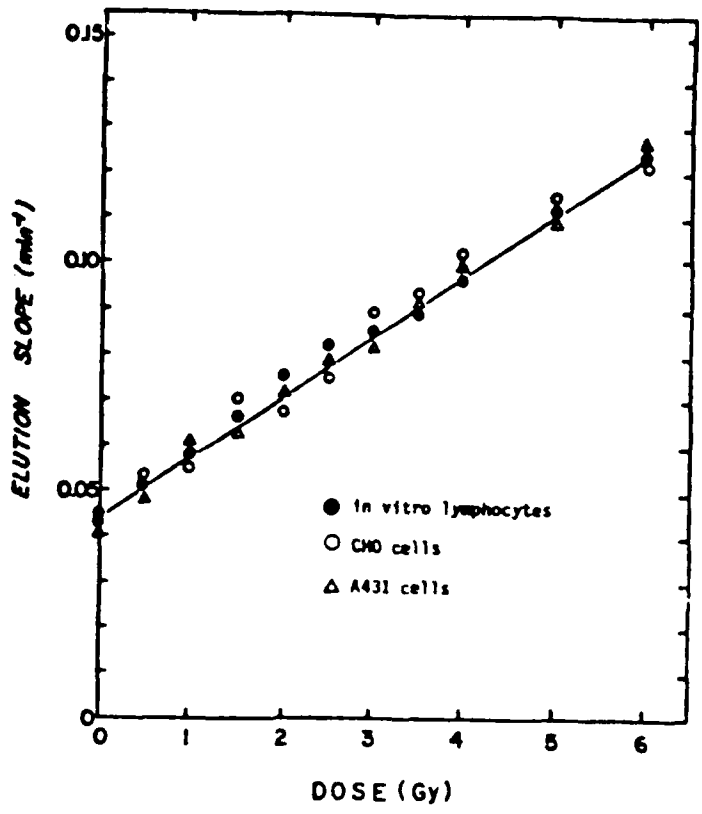

Figure 13. Dose response relationship measured for DNA strand breaks in CHO cell, human squamous carcinoma A431 cell, and in vitro mouse lymphocytes.

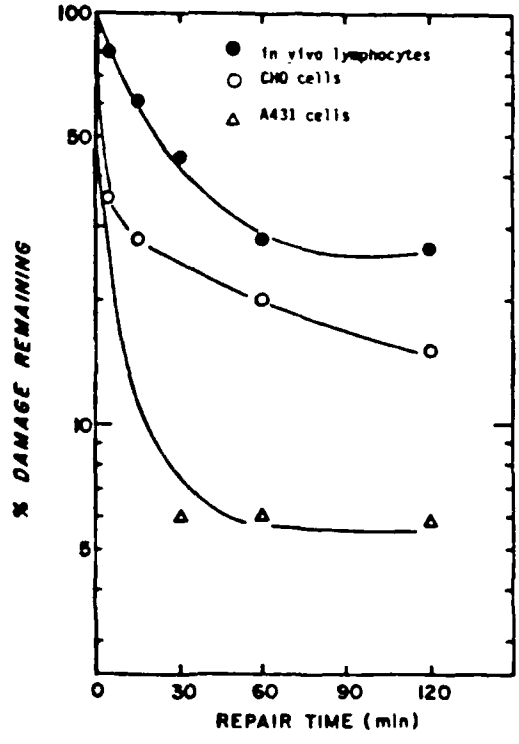

Figure 14. Comparison of DNA strand break rejoining in CHO cells, A431 cells, and in vitro mouse lymphocytes.

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